Application of Random Amplified Polymorphic DNA Markers for Genetic Diversity Assessment of Pomegranate (*Punica granatum* L.) Cultivars in Duhok Governorate – Kurdistan of Iraq

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***Abstract*–Random amplified polymorphic DNA (RAPD) markers were used to investigate the genetic diversity among 13 cultivars of pomegranate cultivated in Duhok governorate, Kurdistan region/Iraq. Unweighed pair-group method average clustering divided the 13 cultivars into three main groups. In RAPD analysis, 12 out of 30 employed random primers showed good amplification and polymorphism on pomegranate samples with a total of 107 bands. The percentage of polymorphic DNA bands ranged from 66.6% (OPX-17) to 100% (OPO-07, OPS-17, and OPE-18, OPP-02, OPK-08, OPL-17, and OPR-01) with an average of polymorphic rate of 94.39. According to matrix coefficient, the lowest genetic similarity was observed between Soormiz and Rash Dendikheer (0.1065). The Dereger and Kapepan populations were most similar ones with coefficient of 0.9374.**

***Index Terms*—Genetic diversity, Random amplified polymorphic DNA markers, Pomegranate.**

1. Introduction

The pomegranate (*Punica granatum* L.) is a woody perennial shrub or small tree (Shahbaz, 2010). It is an edible fruit tree and has been known as the richest dietary sources of antioxidant phenolics and anthocyanins. There is a wide variation among pomegranate genotypes in terms of these compounds (Ozgen, et al., 2008).

Pomegranate is a suitable crop for cultivation in arid and semi-arid regions it has also been considered as an important commercial fruit crop and is extensively cultivated in parts of Asia, North Africa, the Mediterranean, and the Middle East (Sarkhosh, et al., 2006). The origin of pomegranate is considered to be in central of Asia (Harlan, 1992), from where it has spread to the rest of the world (Levin, 1994; Verma, et al., 2010).

Using a sensitive and credible molecular, such as amplified fragment length polymorphism (AFLP), simple sequence repeat, and inter-SSR to detect the DNA variation and to identify the pomegranate germplasm, have become increasingly important way to help breeders and nurserymen with the selection and propagation of a cultivar (Nemati, et al., 2012).

The random amplified polymorphic DNA (RAPD) marker has been used to identify genetic diversity of many different plant species; the use of this reliable marker in pomegranate is limited (Dalal, 2013). The objective of this study is to characterize 13 pomegranate cultivars from the Duhok governorate in Kurdistan region/Iraq by RAPD markers, which will help breeders in parental selection in a pomegranate cultivar-breeding program.

II. Materials and Methods

A. Plant Material

Thirteen different cultivars of pomegranate were selected in Duhok governorate Kurdistan/Iraq. The cultivars names were Soormiz, Rash Dendikheer, Trshigalak, Trshinagala, Dereger, Shreennavekraq, Msafik, Mlese, Melsesharman, Nana (roman Zena), Kapepan, Zaxoly, and Henarok.

B. DNA Extraction and RAPD Analysis

DNA from fresh leaves was extracted following the protocol of Weigand, et al. (1993) using cetyltrimethyl ammonium bromide Protocol. The quantity and quality of isolated genomic DNA was determined using agarose gel (1% [w/v]) electrophoresis and a nanodrop spectrophotometer.

An initial screening with 30 primers (10-mers) from the kits OPA, from Operon Technologic was carried out.

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12 informative primers were selected due to their ability to produce polymorphic, unambiguous and stable RAPD markers (Table I).

RAPD amplification was performed in a 25 µl reaction volume containing 50-100 ng genomic DNA, ×10 buffer (10 mM Tris-Hcl. pH 8.3, 50 mM KCl. 2 mM MgCl), 2.5 mM dNTPs, 10 pMOL of single 10-base primer and 1U *Taq* DNA polymerase (Sigma, USA). The thermocycler was programed as follows; initial cycle of 5 min at 94°C followed by 40 cycles of 1 min at 94°C, an annealing temperature of 36°C for 1 min, extension step of 2 min at 72°C, and a final extension step of 10 min at 72°C. Polymerase chain reaction products were separated by gel electrophoresis on 2% agarose gels with ×1 Trisma base, boric acid, ethylenediaminetetraacetic acid buffer, at 70 V/cm for 2 h. The gel was stained with 0.25 µg/ml ethidium bromide and stained gels were visualized by ultraviolet transilluminator and photographed. The molecular weights of bands were estimated using standard molecular markers.

C. Data Analysis

Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. The data then entered into NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), Version 2.1 (Applied Biostatistics) program (Rohlf, 1993) using the program editor. The data were analyzed using Similarity for Qualitative Data routine to generate genetic similarity index (Nei and Li, 1979).

III. Result and Discussion

Thirteen selected cultivars were genotyped by RAPD markers. 12 informative primers were selected due to their ability to produce polymorphic RAPD markers (Fig. 1).

The range of the dissimilarity matrix obtained varied between 0.106 and 0.937 (Table II); the lowest genetic similarity was observed between Soormiz and rash Dendikheer cultivars (0.106) suggesting their close relatedness. The Dereger and Kapepan cultivars were most similar ones with coefficient of 0.937. The remaining cultivars shared intermediate genetic distance values. Low genetic distances between cultivars supported the inbreeding and low levels of genetic variation.

Genetic distances based on RAPD data and supported by the resulting dendrogram shown in Fig. 2. Three main groups could be identified. The first one is made up of the following cultivars, soormiz, rash Dendikheer, Dereger, Shreennafikreq, Zaxoly, Henarok, and Trshigalak. The second group consisted of Trshinegalak, Mlese, Melse Shaman and Msafik. The third group concluded of nano (romanzena) and Kapepan. The level of the genetic diversity highly correlated with the sample size; therefore, it would be worth mentioning that the used sample size was small in this study. Furthermore, another reason for the low genetic diversity could be due to the vegetative propagation (Behzadi Shahrbabaki, 1997). The results of this study are also in agreement with other studies in the Tunisian pomegranate using the same marker such as RAPD (Hasnaoui, et al., 2010), or different markers such as AFLP (Jbir, et al., 2008).

These primers generated a total of 107 RAPD bands (Table III); the percentage of polymorphic DNA bands ranged from 66.6% (OPX-17) to 100% (OPO-07, OPS-17, OPE-18, OPP-02, OPK-08, OPL-17, and OPR-01) with an average of polymorphic rate of 94.39, indicating a high degree of polymorphism among these pomegranate cultivars,

TABLE I
Represent the Operon Primers and their Sequences

Primer name	Sequence '5-3'
OPE-18	GGACTGCAGA
OPH-07	CTGCATCGTG
OPK-08	GAACACTGGG
OPL-17	AGCCTGAGCC
OPO-07	CAGCACTGAC
OPP-02	TCGGCACGCA
OPP-04	GTGTCTCAGG
OPQ-05	CCGCGTCTTG
OPR-01	TGCGGGTCCT
OPS-17	TGGGGACCAC
OPV-19	GGGTGTGCAG
OPX-17	GACACGGACC

TABLE II
Present Genetic Dissimilar it Coefficient Matrix of the Some Cultivars of *P. granatum*

Soormiz	0.000				
Rash dendikheer	0.106	0.00			
Trshigalak	0.392	0.337	0.000		
Trshinagala	0.687	0.792	0.483	0.000	
Dereger	0.308	0.322	0.278	0.492	0.000
Shreennavekrq	0.217	0.223	0.337	0.560	0.147
Msafik	0.821	0.814	0.722	0.508	0.684
Mlese	0.660	0.676	0.670	0.309	0.570
Melsesharman	0.579	0.616	0.573	0.246	0.489
Roman zena	0.676	0.743	0.839	0.576	0.799
Kapepan	0.699	0.689	0.928	0.840	0.937
Zaxoly	0.351	0.389	0.448	0.502	0.330
Henarok	0.339	0.307	0.469	0.644	0.296

P. granatum: Punica granatum

TABLE III
Represent the Total Number of Bands, Number of Polymorphism, and Polymorphism Rate of Pomegranate Cultivars

Primer name	Total number of bands	Number of polymorphic bands	Polymorphic percentage
OPE-18	7	7	100
OPH-07	10	9	90
OPK-08	8	8	100
OPL-17	14	14	100
OPO-07	10	10	100
OPP-02	8	7	87.5
OPP-04	7	6	85.7
OPQ-05	7	6	85.07
OPR-01	11	11	100
OPS-17	12	12	100
OPV-19	10	9	90
OPX-17	3	2	66.6
Total	107	101	94.39

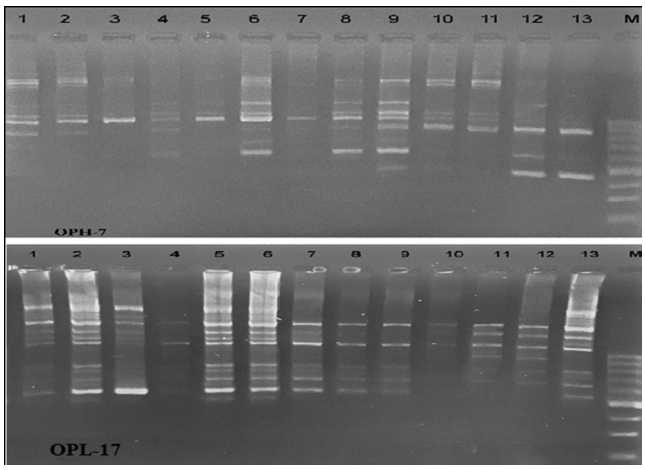

Fig. 1. Random amplified polymorphic DNA patterns representing examples of amplification produced using primers OPH-07 and OPL-17 performed on 2% agarose gel electrophoresis at 70 volt/cm for 2 h. Lane M represent λ DNA, Lanes 1 through 13 refer to pomegranate cultivars: Soormiz, Rash Dendikheer, Trshigalak, Trshinagala, Dereger, Shreennavekraq, Msafik, Mlese, Melsesharman, Nana (Roman Zena), Kapepan, Zaxoly, and Henarok

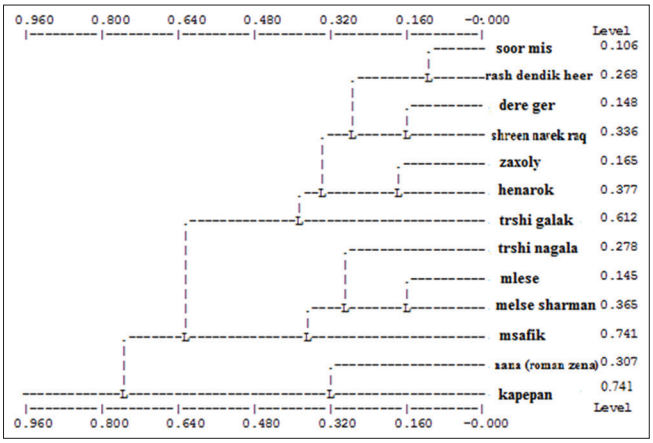

Fig. 2. A dendrogram neighbor joining tree representing the genetic relationships among pomegranate cultivars

which was greater than those reported for Iranian (Sarkhosh, et al., 2006; Zamani, et al., 2007) and Turkish (Durgac, et al., 2008) pomegranate cultivars, which were 57.3%, 57.0% and 22.0%, respectively, using RAPD markers.

The number of bands generated per primer was 8.92. In a study of Hasnaoui, et al. (2010), using four RAPD primers to estimate the genetic diversity among some pomegranate cultivars a total of 29 bands have been generated with a mean of 7.25 per primer.

Based on the pairwise analysis of the amplification products which been obtained with the 12 tested RAPD primers, all the tested pomegranate cultivars showed different relationships. Close genetic similarity was found in some of the cultivars analyzed as shown by high values of similarity index. This richness in genetic diversity could be the result of long-term evolution, and adapt to various environmental conditions (Ercisli, et al., 2011). However, RAPD marker revealed high genetic variability among pomegranate genotypes and it could be used for identification and characterization of pomegranate genotypes. In addition, complete comprehension of the genetic diversity within cultivars would contribute to a more efficient use of germplasm in plant breeding programs. Furthermore, it is essential to investigate other molecular markers linked closely to the morphological traits of pomegranate in the future. The *P. granatum* cultivars selected from Duhok governorate displayed very rich genetic variations.

IV. Conclusion

The results demonstrated that RAPD profiles are valuable tools with the great potential for classifications of pomegranate cultivars. In addition, complete comprehension of the genetic diversity within cultivars would contribute to a more efficient use of germplasm in plant breeding programs. Furthermore, it is essential to investigate other molecular markers linked closely to the morphological traits of pomegranate in the future.

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